EV_71_BsAI_BsmbI_free

Created: 2/16/2022, 7:32:14 PM
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Component Fragments

Name	Length	Produced by	5' End	3' End
PCRFragment_2	2086	PCR	Fwd Primer (auto)	Rev Primer (auto)
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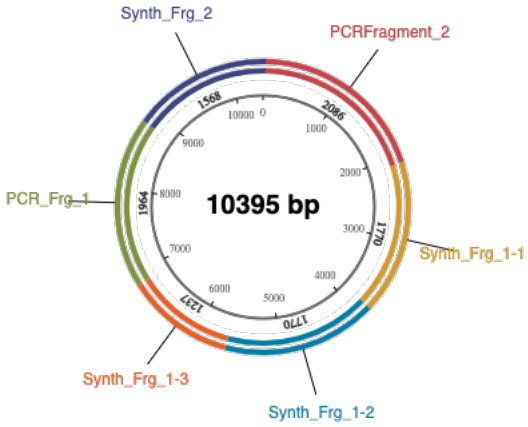
Synth_Frg_1-2	1800	Synthetic	---	---
Synth_Frg_1-3	1267	Synthetic	---	---
PCR_Frg_1	1994	PCR	Fwd Primer (auto)	Rev Primer (auto)
Synth_Frg_2	1628	Synthetic	---	---



Notes

- For assemblies of 4 or more fragments, we recommend using overlaps of at least 25 bp when using NEBuilder.
- A 60 minute reaction is recommended for the assembly of more than 3 fragments.

Required oligos

Name	Primer 5' (overlap/spacer/ANNEAL) 3'	Len	%GC	3' %GC	3' Tm	3' Ta
PCRFragment_2_fwd	**AGCAGACAAGCCCGTCAG**	18	61	61	67.9	64.4
PCRFragment_2_rev	**TTGGTTTGTGCCAGGTTTC**	19	47	47	63.4	64.4
PCR_Frg_1_fwd	**TGAGAATGACATTTGGGC**	18	44	44	59.4	58.7
PCR_Frg_1_rev	**ATAAGTCGTGTCTTACCG**	18	44	44	57.7	58.7

Build Settings

Property	Value
Product/Kit	#E5520 NEBuilder HiFi DNA Assembly Cloning Kit
Minimum Overlap	20 nt
Minimum Overlap Tm	48 °C
Circularize	Yes
PCR Polymerase/Kit	Q5 High-Fidelity DNA Polymerase
PCR Primer Conc.	500 nM
Min. Primer Length	18 nt

Assembled Sequence

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#ACCESSION   .
#VERSION     .
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#SOURCE      synthetic DNA construct
#  ORGANISM  synthetic DNA construct
#REFERENCE   1  (bases 1 to 10395)
#  AUTHORS   .
#  TITLE     NEBuilder-generated Construct
#  JOURNAL   Exported  16-FEB-2022 from NEBuilder https://nebuilder.neb.com
#COMMENT     NEBuilder-generated oligos (UPPERCASE = gene-specific, lowercase = overlap)
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#COMMENT     PCRFragment_2_rev: TTGGTTTGTGCCAGGTTTC
#COMMENT     PCRFragment_2_rev 3'Tm: 63.4  3'Ta: 64.4
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#COMMENT     PCR_Frg_1_rev: ATAAGTCGTGTCTTACCG
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